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Localization of the ICF Syndrome to Chromosome 20 by Homozygosity Mapping

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Summary

Immunodeficiency in association with centromere instability of chromosomes 1, 9, and 16 and facial anomalies (ICF syndrome) is a rare autosomal recessive disorder. ICF patients show marked hypomethylation of their DNA; undermethylation of classical satellites II and III is thought to be associated with the centromere instability. We used DNA from three consanguineous families with a total of four ICF patients and performed a total genome screen, to localize the ICF syndrome gene by homozygosity mapping. One chromosomal region (20q11-q13) was consistently found to be homozygous in ICF patients, whereas all healthy sibs showed a heterozygous pattern. Comparison of the regions of homozygosity in the four ICF patients localized the *ICF* locus to a 9-cM region between the markers D20S477 and D20S850. Analysis of more families will be required, to refine the map location further. Isolation of the gene associated with the ICF syndrome not only will give insight into the etiology of the ICF syndrome but will also broaden our understanding of DNA methylation processes.

Introduction

Immunodeficiency in association with centromere instability of chromosomes 1, 9, and 16 and facial anomalies (ICF syndrome [MIM 242860]) is an extremely rare condition first described nearly 20 years ago (Hultén 1978; Tiepolo et al. 1979); only 20 patients have been reported in the literature (Smeets et al. 1994; Brown et al. 1995; Franceschini et al. 1995). ICF syndrome is characterized by the presence of an immunodeficiency—either a combined immunodeficiency or a deficiency of a single immunoglobulin—in combination with facial dysmorphisms, including epicanthic folds, telecanthus, a flat nasal bridge, macroglossia with a protruding tongue, and mild micrognathia. ICF patients generally suffer from recurrent and prolonged respiratory infections that are due to their immunodeficiency. All ICF patients have instability of pericentric heterochromatin of chromosomes 1, 9, and/or 16. DNA methylation studies in ICF patients showed hypomethylation of classical satellites II and III, which are major components of constitutive heterochromatin (Jeanpierre et al. 1993; Schuffenhauer et al. 1995; Miniou et al. 1997).

The existence of several families with several affected sibs suggests a genetic predisposition to ICF (Valkova et al. 1987). In addition, the majority of ICF patients have been born from consanguineous marriages, suggesting that ICF is transmitted as an autosomal recessive trait (Valkova et al. 1987). The extremely rare occurrence of the ICF syndrome and the existence of affected, inbred individuals make the localization of the gene underlying this disorder a suitable target for homozygosity mapping. This mapping approach is based on the inheritance, from a common ancestor, of two identical copies of the disease locus, by the affected inbred child—that is, homozygosity by descent (HBD) (Lander and Botstein 1987). Homozygosity mapping has been used to localize several recessive disease genes, including Rogers syndrome (Neufeld et al. 1997), Hallervorder-Spatz syndrome (Taylor et al. 1996), and urofacial (Ochoa) syndrome (Wang et al. 1997).

The size of the region around a disease locus that shows HBD in an affected inbred individual is determined by the number of meiotic steps that separate the affected individual from the common ancestor who car-

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ried a single copy of the disease chromosome. A relatively large region of ~30 cM around the disease locus can be expected to show HBD when the patient is the product of a first-cousin marriage (Terwilliger et al. 1997). In contrast, a separation by 12 meiotic steps yields, on average, a region of HBD of only 16 cM around a disease locus. Consequently, as the number of meiotic steps increases, the length of the conserved ancestral segment that will be homozygous by descent becomes smaller. When the conserved fragments become too small, they may be easily missed if markers with an average spacing of 10 cM are used, and a higher density of markers will be needed.

We used homozygosity mapping in three consanguineous ICF families. One ICF family with two affected brothers and common ancestors six generations ago (figure 1) was initially used to perform a genomewide scan to find regions that were homozygous and shared between the two affected sibs. Six different chromosomal regions were identified, and the remaining two consanguineous families were used to confirm and delimit a region on chromosome 20 that was homozygous in all four affected inbred individuals. Comparing the regions of homozygosity in the four ICF patients maps the locus responsible for ICF syndrome (*ICF*) to a 9-cM interval on 20q11-q13.

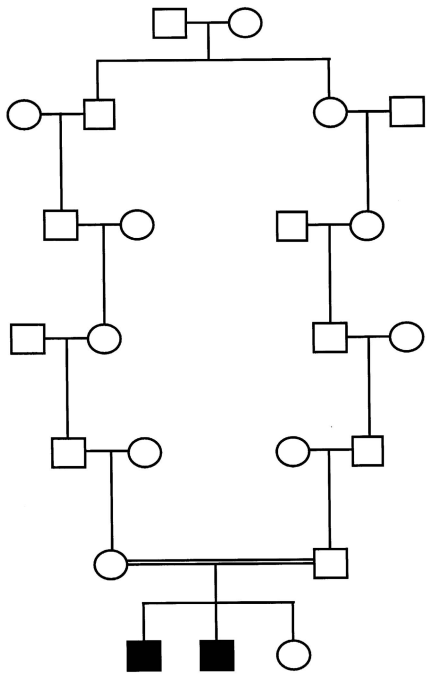

Figure 1 Pedigree ICF family 1. The parents go back to a common ancestor 5 generations ago.

Subjects and Methods

Subjects

We investigated three families containing four ICF patients and seven unaffected family members. All four patients are the offspring of consanguineous unions. The Dutch family 1 was previously reported as an apparently nonconsanguineous family (Smeets et al. 1994). Extensive genealogical studies identified, for the parents, a common ancestor five generations ago (figure 1). The clinical and laboratory data for patient P1 were described by Smeets et al. (1994). The diagnosis of ICF in his brother, P2, born in 1994, was made soon after birth, by means of cytogenetic studies. Treatment for agammaglobulinemia was started at 6 weeks of age. Patient P2 is doing well and has never had serious infections.

In the two Turkish families 2 and 3, there had been first-cousin marriages. Patient P3 of family 2, born in 1996, was admitted to hospital for respiratory infections and diarrhea. Cytogenetic studies showed the typical ICF abnormalities. She displayed minor facial abnormalities. Immunological studies showed agammaglobulinemia, for which she received immunoglobulin substitution. Patient P4 of family 3, born in 1983, was diagnosed as having ICF syndrome, by means of cytogenetic studies. Despite treatment for agammaglobulinemia, she suffered from recurrent respiratory infections. At the age of 11 years, she died from sepsis after surgery for gastroesophageal reflux.

Marker Analysis

DNA was extracted from 20 ml blood by means of standard procedures (Miller et al. 1988). The genome screen was performed with markers from the Marshfield screening set, v6 (Center for Medical Genetics). These markers have an average spacing of 10 cM and an average heterozygosity of ~0.75. Reverse primers were labeled with either 6-FAM, HEX, or TET fluorescent dyes (Isogen Biosciences), which allowed analysis of a high density of markers on an automated ABI377 DNA sequencer. PCR reactions were performed in an ABI877 in a 10-μl volume containing 30 ng template DNA, 25 ng each oligonucleotide primer, 200 mM each dNTP, and 0.4 units Amplitaq Gold (Perkin Elmer), in 1 × PCR buffer II with 2.5 mM $MgCl_2$ (Perkin Elmer). DNA was initially denatured at 94°C for 10 min and was then subjected to 32 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension step of 10 min at 72°C. One microliter of the PCR reaction was mixed with 1 μl formamide, 0.25 μl GS-500 size standard (Perkin Elmer), and 0.25 μl Blue Dextran (Perkin Elmer) and separated on nondenaturing 5% polyacrylamide gels on an ABI377. Gel images were analyzed by use of GeneScan 2.1 (Perkin Elmer). Allele sizes of the

individual markers were determined by use of GenoTyper 2.0 (Perkin Elmer).

CEPH reference samples (1331-02 and 1347-02) were included, to determine the appropriate size of the alleles. Allele frequencies for the Dutch population in this and other studies in our laboratory are generally in good agreement with published allele frequencies. For the Turkish population and, in particular, for isolated communities in Turkey, accurate allele frequencies are unavailable.

Homozygosity Mapping

A total genome screen using 338 markers was performed on DNA from four members of family 1 (M1, F1, P1, and P2). For each marker, we determined whether the two affected sibs (P1 and P2) were heterozygous or homozygous for the same marker alleles. All genomic regions in which two or more consecutive markers showed homozygosity by state were studied further by screening an additional ICF family (family 2) and an isolated patient (family 3) with the same markers.

Localization of the ICF Locus to 20q11-q13

To determine the region of homozygosity on chromosome 20 more precisely, we used additional markers: D20S111, D20S200, D20S850, D20S108, D20S119, D20S888, and D20S887. The map positions of these markers with respect to the chromosome 20 markers already analyzed during the genome screen are depicted in figure 2. Multipoint LOD scores were calculated by use of the computer program MAPMAKER/HOMOZ (Kruglyak et al. 1995), with allele frequencies as published for Caucasians. In addition, for the Turkish families 2 and 3, multipoint LOD scores were also calculated with a lower threshold of 0.25 for all allele frequencies. This threshold corresponds to the weighted average of allele frequencies for markers with heterozygosity equal to 0.75.

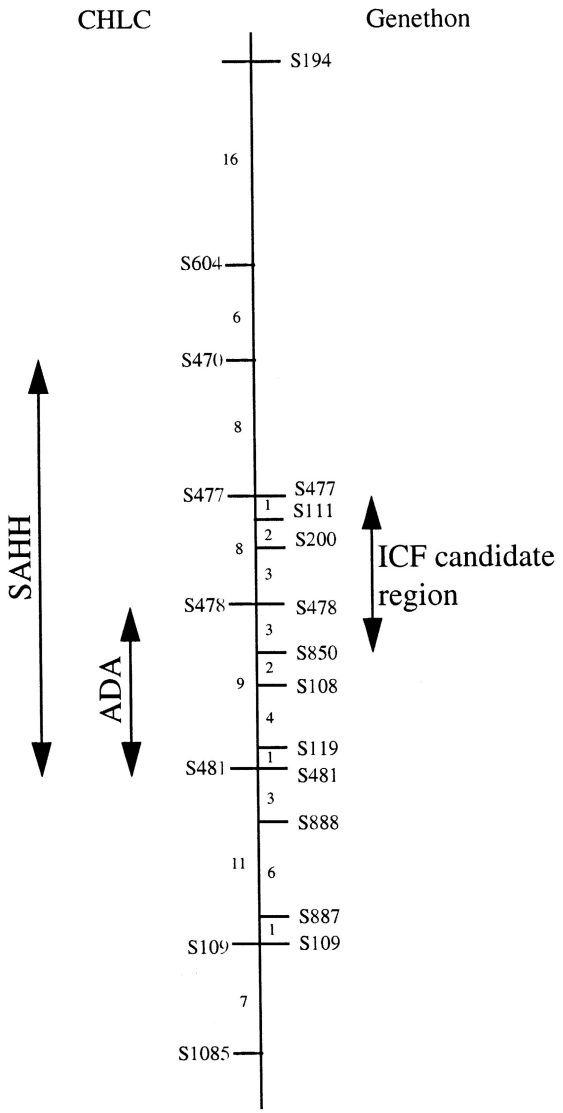

Figure 2 Genetic map and distances of the chromosome 20 markers. The location of the ICF gene is based on the region of homozygosity in all four patients. The distances between the markers are in centimorgans and are based on previously published data (Cooperative Human Linkage Center map: Buetow et al. 1994; Généthon map: Dib et al. 1996) or were extracted from the CEPH database.

Results

Genome Screen

Initially, DNA from two affected ICF sibs from family 1 was analyzed with 338 markers (covering ~90% of the genome), yielding 45 markers (13%) that showed homozygosity by state in both affected sibs. The homozygous markers were scattered throughout the genome. However, clusters of two or three adjacent homozygous markers were identified on 2p, 6q, 8q, 10p, 14q, and 20q. These six regions were considered to be putative ICF candidate regions needing further investigation. Three different approaches were followed to confirm or exclude the presence of the ICF locus for each of these six loci: (i) typing two additional families (families 2 and 3), (ii) haplotype analysis to determine whether the regions were also identical by descent, and (iii) typing the healthy sib from family 1.

Further Examination of ICF Candidate Regions and Mapping the ICF Locus to 20q

Family 2 was typed for markers from the six homozygous regions observed in family 1 together with two flanking markers for each region (25 markers total). Of these, 11 markers, including 4 consecutive markers from 14q (D14S53, D14S606, D14S610, and D14S617) and

4 consecutive markers from 20q (D20S470, D20S477, D20S478, and D20S481), showed homozygosity by state in patient P3. However, haplotype analysis excluded 14q as a likely location for the ICF locus. Although the two affected sibs from family 1 (P1 and P2) showed homozygosity by state for marker alleles at D14S606 and D14S610, their mother was homozygous for these markers. For informative markers flanking this interval, the two affected sibs did not share their maternal allele (data not shown).

For family 3, only DNA from the proband was available. Analysis of chromosome 20 markers showed homozygosity for four successive markers from 20q (D20S478, D20S481, D20S109, and D20S1085). None of the three healthy sibs (families 1 and 2) that were included showed homozygosity for this 20q region.

Interval Mapping

Further investigation with seven additional polymorphic markers and a comparison of the regions of homozygosity observed in the four ICF patients placed the *ICF* locus, in individual P4, telomeric to D20S477 and, in individuals P1 and P2, centromeric to D20S850.

Table 1

Multipoint LOD Scores between ICF Syndrome and Chromosome 20 Markers

	Multipoint LOD Score			
Marker[a]	Family 1	Family 2	Family 3	Total
D20S194*	−∞	−1.57	−1.878	−∞
D20S604*	−∞	−1.60	.13	−∞
D20S470*	2.81	1.34	−1.92	2.23
D20S477*	3.21	1.45	−1.97	2.68
D20S111	3.20	1.45	.86	5.50
D20S200	3.17	1.45	1.10	5.72
D20S478*	3.00	1.45	1.17	5.62
D20S850	.43	1.45	1.19	3.07
D20S108	.43	1.45	1.19	3.07
D20S119	1.30	1.45	1.19	3.94
D20S481*	.43	1.45	1.19	3.07
D20S888	.43	−2.80	1.19	−1.17
D20S887	.43	−2.49	1.18	−.88
D20S109*	.43	−99	1.18	−97.39
D20S1085*	.37	−.17	1.11	1.34

[a] The markers originally analyzed in the total genome screen are marked with an asterisk.

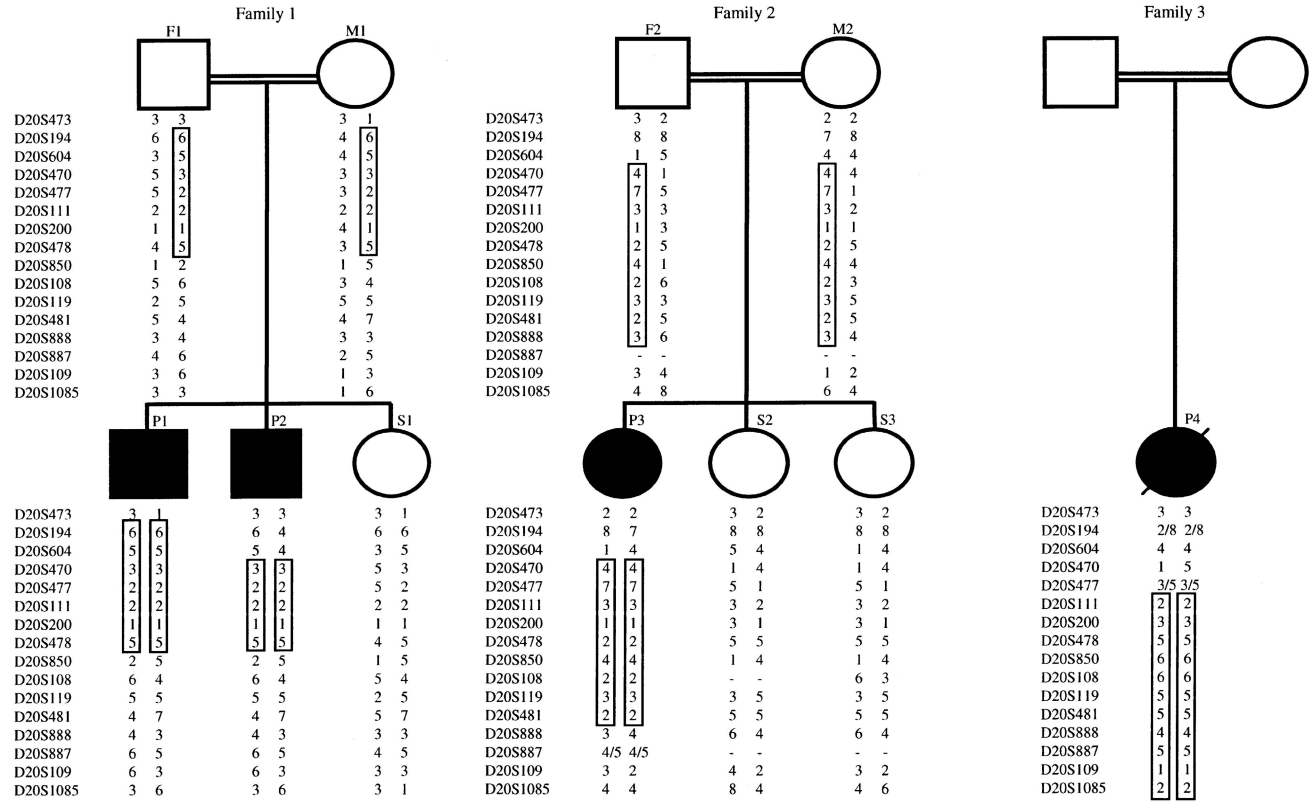

Figure 3 Simplified pedigrees and genotypes in the region of homozygosity. Families 2 and 3 are first-cousin marriages. Dashes (–) indicate genotypes that were not determined. The regions of homozygosity in the affected children are boxed.

Therefore, the interval between D20S477 and D20S850 delimits the *ICF* locus to a region of $\leqslant 9$ cM (figure 3). This chromosomal location by haplotype analysis was in agreement with the results of the multipoint LOD score analysis (see table 1 and figure 4). The peak multipoint LOD scores ($Z > 5$) were obtained at D20S111 ($Z = 5.50$), D20S200 ($Z = 5.72$), and D20S478 ($Z = 5.62$). The peak multipoint LOD scores were derived mainly from the Dutch family 1 ($Z = 3.20$, 3.17, and 3.00, respectively). The multipoint LOD score results were not sensitive to the specified allele frequencies (maximum reduction in multipoint LOD score 0.07; data not shown).

Two possible candidate genes from the 20q region were investigated further: adenosine deaminase (ADA) and *S*-adenosylhomocysteine hydrolase (ACHY or SAHH). Deficiency of ADA or SAHH can lead to DNA hypomethylation, a phenomenon that has been demonstrated in ICF patients (Schuffenhauer et al. 1995; Miniou et al. 1997). The enzyme activities of ADA and SAHH were measured in lymphocytes of three ICF patients (P1, P2, and P3) but appeared to be normal (data not shown). Therefore, ICF is not caused by deficiency of ADA or SAHH.

Discussion

The locus for the ICF syndrome was localized by homozygosity mapping to a 9-cM region on chromosome 20, between the markers D20S477 and D20S850, in four ICF patients. Further delimitation of this region will require more families and the use of additional polymorphic DNA markers. However, the scarcity of ICF families is the major limiting factor.

Two enzymes, encoded by genes in the 20q region, that are possibly involved in the methylation of DNA were investigated: ADA and ACHY (or SAHH). ADA and SAHH deficiency can lead to increased intracellular concentrations of deoxy-adenosine and *S*-adenosyl-L-homocysteine, respectively. These metabolites are known inhibitors of crucial methyl transfer reactions by *S*-adenosyl-L-methionine, involved in DNA methylation (Ueland 1982). The normal enzyme activities of both ADA and SAHH in our ICF patients excluded their genes

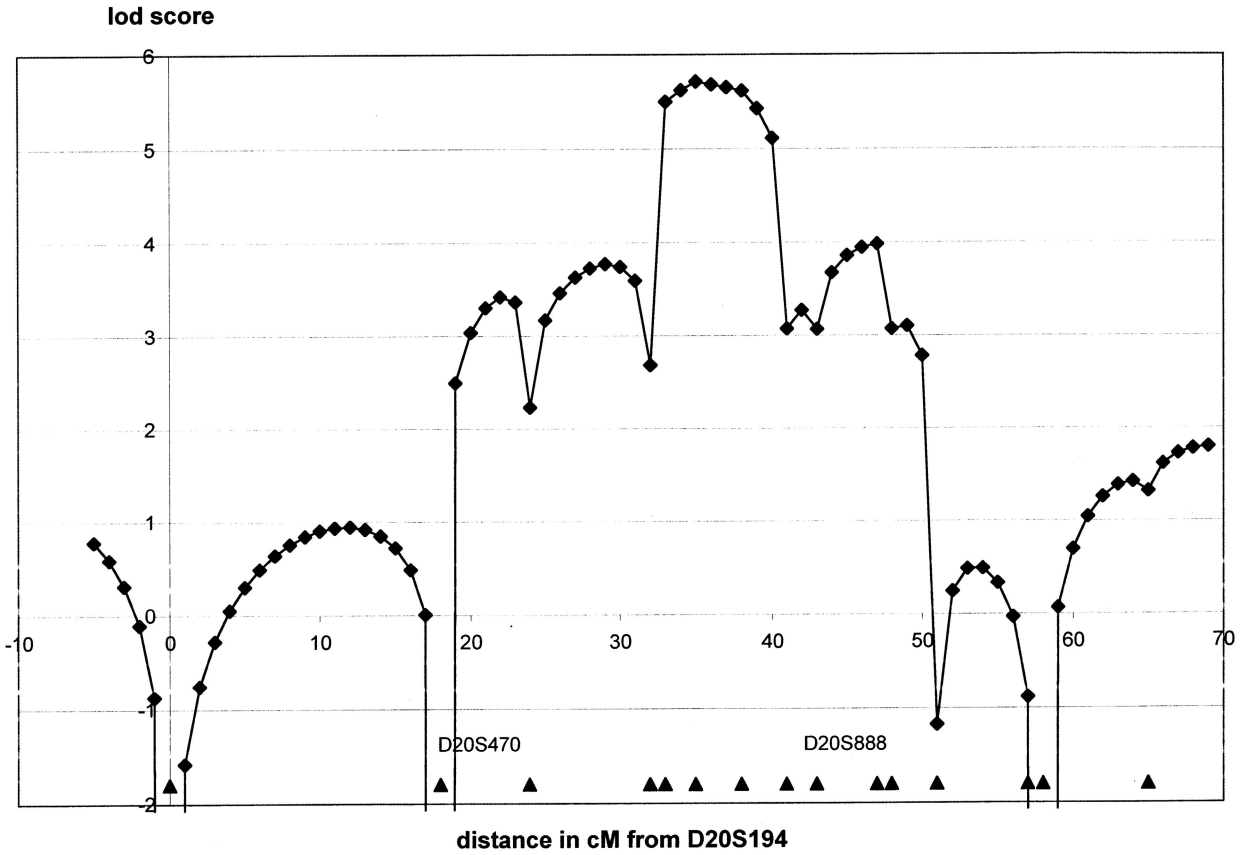

Figure 4 Results of linkage analysis between the ICF locus and 16 markers on chromosome 20q11-q13. The positions of the markers are indicated by triangles.

as candidates for ICF. Since, in the mouse, the *lethal nonagouti* (a^x) mutation is a recessive lethal mutation, we could expect the complete loss of SAHH activity to be lethal in humans. This mouse mutation is a deletion of the *achy* gene, which results in complete loss of SAHH activity (Miller et al. 1994).

The 20q region defined by our mapping studies is still too large to make positional cloning feasible. However, several ESTs of unknown function have been assigned to the region. However, a search for other genes encoding enzymes involved in DNA methylation is attractive, as this could provide further insight into fundamental biological processes. Our results confirm the effectiveness of homozygosity mapping as a tool for gene localization of rare inbred disorders.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics (for markers used in the genome screen)

Cooperative Human Linkage Center, http://www.chlc.org (for genetic map and distances between chromosome 20 markers)

Généthon, CEPH genotype database http://www.cephb.fr (for genetic map and distances between chromosome 20 markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nim.nih.gov/Omim (for ICF syndrome [MIM 242860])

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